

WHAT IS CLAIMED IS:

1                   1.       A method of testing the integrity of primers in a multiplex  
2   amplification reaction, the amplification reaction comprising primers sufficient to amplify at  
3   least two different target sequences, the method comprising,  
4                    providing in a mixture the primers and a single-stranded polynucleotide  
5   sequence comprising the sequences of the primers, subsequences of the primers at least five  
6   nucleotides long, or complements of the sequences of the primers;  
7                    amplifying the polynucleotide sequence; and  
8                    detecting the presence or absence of the amplified polynucleotide, thereby  
9   testing the integrity of the primers in the amplification reaction.

1                   2.       The method of claim 1, wherein the target sequences are less than 50%  
2   identical to each other.

1                   3.       The method of claim 1, wherein the single-stranded polynucleotide  
2   sequence is provided by denaturing a double-stranded polynucleotide.

1                   4.       The method of claim 1, wherein the single-stranded polynucleotide  
2   sequence is a synthetic single-stranded polynucleotide.

1                   5.       The method of claim 1, wherein the single-stranded polynucleotide  
2   sequence comprises the primer sequences.

1                   6.       The method of claim 1, wherein the single-stranded polynucleotide  
2   sequence comprises subsequences of the primers at least five nucleotides long.

1                   7.       The method of claim 1, wherein the single-stranded polynucleotide  
2   sequence comprises all subsequences of the primers that are nine nucleotides long.

1                   8.       The method of claim 1, wherein the single-stranded polynucleotide  
2   comprises at least two subsequences of each primer, wherein the combination of the at least  
3   two subsequences contain every nucleotide of the primer sequence.

1                   9.       The method of claim 1, wherein the single-stranded polynucleotide  
2   sequence comprises two subsequences of a primer sequence and at least the last two

3 nucleotides of a first subsequence are identical to the first at least two nucleotides of a second  
4 subsequence.

1 10. The method of claim 9, wherein at least the last five nucleotides of the  
2 first subsequence are identical to at least the first five nucleotides of the second subsequence.

1 11. The method of claim 1, wherein the mixture comprises at least a first,  
2 second, and third primer and the single-stranded polynucleotide sequence comprises the  
3 sequences of the at least first, second and third primer or subsequences at least five  
4 nucleotides long of the at least first, second and third primers.

1 12. The method of claim 1, wherein the mixture comprises primers  
2 sufficient to amplify at least three target sequences.

1 13. The method of claim 1, wherein the amplification of the target  
2 sequences is performed in the same reaction as the amplification of the single-stranded  
3 polynucleotide sequence.

1 14. The method of claim 1, wherein the mixture comprises a first primer  
2 pair and the single-stranded polynucleotide sequence comprises sequences, or complement  
3 thereof, of primers of the first primer pair oriented such that the first primer pair is capable of  
4 amplifying the remaining primer sequences, or subsequences thereof, in the single-stranded  
5 polynucleotide.

1 15. The method of claim 14, wherein the mixture comprises at least a  
2 second primer pair comprising a forward and a reverse primer, wherein the single-stranded  
3 polynucleotide sequence comprises sequences or subsequences of the at least second primer  
4 pair oriented such that the reverse primer sequence or subsequence is closer to the 5' end of  
5 the polynucleotide sequence than the forward primer sequence or subsequence.

1 16. The method of claim 15, wherein the single-stranded polynucleotide  
2 sequence comprises subsequences of the primers at least five nucleotides long.

1 17. The method of claim 15, wherein the single-stranded polynucleotide  
2 sequence comprises all subsequences of the primers that are nine nucleotides long.

1 18. A reagent kit, comprising

2 i. amplification reagents comprising primers sufficient to amplify at least  
3 two different target sequences;

4 ii. a polynucleotide sequence comprising the sequences of the primers or  
5 subsequences of the primers at least five nucleotides long; and

6 iii. at least one probe for detecting the polynucleotide sequence.

1 19. The reagent kit of claim 18, wherein the polynucleotide sequence is  
2 single-stranded.

1 20. The reagent kit of claim 18, wherein the polynucleotide sequence  
2 comprises the primer sequences.

1 21. The reagent kit of claim 18, wherein the polynucleotide sequence  
2 comprises subsequences of the primers at least five nucleotides long.

1 22. The reagent kit of claim 18, wherein the amplification reagents  
2 comprise a first primer pair and the single-stranded polynucleotide sequence comprises  
3 sequences, or complement thereof, of primers of the first primer pair oriented such that  
4 primer pair is capable of amplifying the remaining primer sequences, or subsequences  
5 thereof, in the single-stranded polynucleotide.

1 23. The reagent kit of claim 22, wherein the amplification reagents  
2 comprise at least a second primer pair comprising a forward and a reverse primer, wherein  
3 the single-stranded polynucleotide sequence comprises sequences or subsequences of the at  
4 least second primer pair oriented such that the reverse primer sequence or subsequence is  
5 closer to the 5' end of the polynucleotide sequence than the forward primer sequence or  
6 subsequence.

1 24. The reagent kit of claim 23, wherein the single-stranded polynucleotide  
2 sequence comprises subsequences of the primers at least five nucleotides long.

1 25. The reagent kit of claim 18, wherein the polynucleotide sequence  
2 comprises all subsequences of the primers that are nine nucleotides long.

1 26. The reagent kit of claim 18, wherein the polynucleotide sequence  
2 comprises two subsequences of a primer sequence and at least the last two nucleotides of a  
3 first subsequence are identical to the first at least two nucleotides of a second subsequence.

1                    27.     The reagent kit of claim 26, wherein at least the last five nucleotides of  
2     the first subsequence are identical to at least the first five nucleotides of the second  
3     subsequence.

1                    28.     The reagent kit of claim 18, wherein the kit comprises at least a first,  
2     second, and third primer and the single-stranded polynucleotide sequence comprises the  
3     sequences of the at least first, second and third primer or subsequences at least five  
4     nucleotides long of the at least first, second and third primers.

1                    29.     The reagent kit of claim 18, wherein the reagent kit comprises a first  
2     primer pair and the polynucleotide sequence comprises sequences of primers of the first  
3     primer pair oriented such that the first primer pair is capable of amplifying the remaining  
4     primer sequences, or subsequences thereof, in the single-stranded polynucleotide.

1                    30.     The reagent kit of claim 18, wherein the kit comprises at least a second  
2     primer pair comprising a forward and a reverse primer, and the single-stranded  
3     polynucleotide sequence comprises sequences or subsequences of the at least second primer  
4     pair oriented such that the reverse primer sequence or subsequence is closer to the 5' end of  
5     the polynucleotide sequence than the forward primer sequence or subsequence.

1                    31.     The reagent kit of claim 30, wherein the single-stranded polynucleotide  
2     sequence comprises subsequences of the primers at least five nucleotides long.

1                    32.     The reagent kit of claim 30, wherein the single-stranded polynucleotide  
2     sequence comprises all subsequences of the primers that are nine nucleotides long.